Multisensory pollution: artificial light at night and anthropogenic noise have interactive effects on activity patterns of great tits (*Parus major*)

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Abstract

Urbanisation is increasing globally at a rapid pace. Consequently, wild species face novel environmental stressors associated with urban sprawl, such as artificial light at night and noise. These stressors have pervasive effects on the behaviour and physiology of many species. Most studies have singled out the impact of just one of these stressors, while in the real world they are likely to co-occur both temporally and spatially, and we thus lack a clear understanding of the combined effect of anthropogenic stressors on wild species. Here, we experimentally exposed captive male great tits (*Parus major*) to artificial light at night and 24h noise in a fully factorial experiment. We then measured the effect of both these stressors on their own and their combination on the amount and timing of activity patterns. We found that both light and noise affected activity patterns when presented alone, but in opposite ways: light increased activity, particularly at night, while noise reduced it, particularly during the day. When the two stressors were combined, we found a synergistic effect on the total activity and the nighttime activity, but an antagonistic effect on daytime activity. The significant interaction between noise and light treatment also differed among forest and city birds. Indeed, we detected a significant interactive effect on light and noise on daytime, nighttime, dusktime and offset of activity of urban birds, but not of forest birds. These results suggest that both artificial light at night and anthropogenic noise can drive changes in activity patterns, but that the specific impacts depend on the habitat of origin. Furthermore, our results demonstrate that co-occurring exposure to noise and light can lead to a stronger impact at night than predicted from the additive effects and thus that multisensory pollution may be a considerable threat for wildlife.

Summary capsule: Anthropogenic light and noise have interactive effects on bird activity patterns, and urban and forest birds differ in their response to these sensory pollutants.

Introduction

Urbanisation is one of the most important global changes and widely recognized as a primary source of modification of the natural environment (*1–3*). More people are now living in urban than rural areas (*4*), and as this figure is projected to increase steadily over the next few decades, this will likely result in progressing urbanisation in most areas of the world, and particularly in developing countries (*5*). Urbanisation poses novel challenges to wild species as organisms are exposed to a suite of environmental factors that are either completely absent or of minor importance in rural and natural areas. Consequently, many studies have revealed profound phenotypic shifts in urban vs rural populations of the same species, which refer to many different traits and span different levels of biological organization (gene expression, hormone secretion, energy metabolism, behavioural traits) (*6–10*).

In the last two decades, research in the field of adaptation to human activities has sought to identify the environmental drivers of such phenotypic shifts, often focusing on disentangling the effects of a single specific anthropogenic factor from all the many others that co-occur in human-dominated landscapes such as cities. Such an approach has been deployed in many correlative studies (*11–16*). Moreover, experimental work is also emerging, with many studies conducted in the field (*17–21*) but also in captivity (*22*, *23*). However, urban-specific environmental factors often co-occur and co-vary (*24*), and it is therefore crucial to study their combined impacts, particularly in cases where such combined impacts differs from the expected impacts based on estimated additive effects (*25*, *26*). This is particularly relevant in cases where multiple environmental stressors influence similar phenotypic traits. Combined effects may be additive (whole being equal to the exact sum of parts), antagonistic (whole being lesser than the sum of parts), or synergistic (whole being greater than the sum of parts) (*27–29*).

Artificial light at night and anthropogenic noise have recently received increasing attention, with studies focusing on the impact of these so-called sensory pollutants on phenotypic traits related to an animal's perception, physiology and behaviour (*26*, *30–33*). The impact of both sensory pollutants has been linked to changes in survival and reproductive performance across a wide range of taxa, including birds (*34*, *35*), mammals (*36*), fish (*37*, *38*) and insects (*39*). Experimental studies have revealed how noise and light pollution influence perceptual processes during foraging (*40*), mating (*41*, *42*) or predator avoidance (*43*, *44*). Furthermore, many studies have assessed how animals cope with these perceptual impacts by adjusting their behaviour (*45–47*) and physiology (*48*), and in few cases their perceptual sensitivities (*49*, *50*). The impacts of light and noise on the activity patterns of birds have been particularly well studied. Correlating levels of artificial night lighting to onset of dawn song has repeatedly revealed that song birds in light-polluted areas start their dawn song earlier (*11*, *51*, *52*), and that the effect of light-pollution varies with the season (*53*, *54*), as well as with species-specific sensitivities to light (*41*, *55*). Experimental exposure in the lab confirmed a causal link between light levels at night and nocturnal and crepuscular activity in several bird species (*56–59*). A recent field experiment, however, has failed to indicate that light pollution can affect onset of dawn song in several bird species (*60*), although the illumination might have been too localized to have an impact as birds could easily roost in nearby dark locations (*46*). Studies on anthropogenic noise, in particular generated by traffic, have revealed that birds start singing in earlier, or may even switch to singing at night in noisier territories (*61*, *62*). Experimental exposure in the field confirmed a causal link between noise levels around sunrise and the onset of dawn singing of a community of bird species (*63*).

Despite the fact that light at night and noise often co-occur, in particular in urbanised areas (*64*), few studies so far have addressed how these two sensory pollutants can influence

each other's impact. In birds, several correlational studies investigated the combined effects of light and noise on activity patterns, with contrasting results. In European robins (*Erithacus rubecola*), daytime noise rather than light at night has been suggested to drive nocturnal singing behaviour (*62*). Later studies contradicted this result in robins and also in other songbirds species, suggesting that light was the strongest predictor of nocturnal activity (*11*, *62*, *65*, *66*). The experimental studies conducted in the field were usually designed to manipulate either one or the other variable, but not both simultaneously (*21*). Moreover, in several cases the levels of the non-manipulated variable was not even measured, thus failing to control for small scale variation in light and noise levels that may have affected the interpretation of the results (*60*, *63*, *67*). In general, all these studies typically ignored testing for potential interaction effects between the two sensory pollutants. The few studies that used a full-factorial experimental exposure to noise and light pollution either yielded inconclusive results or did not specifically test whether the combined impact is additive, antagonistic or synergistic (*28*, *29*, *68*).

Here we aim to combine knowledge and insights from studies on both light and noise pollution to address whether these two sensory stimuli can influence each other's impact. Specifically, we asked whether the combined impact of noise and light was additive, synergistic or antagonistic (Figure 1). We collected male great tits (*Parus major*) from both urban and rural areas and kept these birds in the lab under controlled noise and light levels. We used birds from different populations to specifically test whether urban birds might have developed a different sensitivity/tolerance to light at night and noise, as previous studies suggested this might be the case (*69*). We exposed each individual to moderate levels of anthropogenic noise and artificial light at night using a balanced, full-factorial, repeated measures design. We automatically scored an animal's activity level by recording the number of perch hops individuals performed in their experimental cage and used this data to calculate

their activity during various parts of the day and night, as well as the onset and offset of their daily cycle.

Materials and methods

Experimental subjects

The experiment was carried out at the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen, The Netherlands between 19th of February and 17th of March 2018. Male great tits were caught in September 2017 in several rural (17 birds) and urban areas (16 birds) throughout the Netherlands (see Suppl. Table 1 for details on sampling locations). Birds were individually housed in cages (90x50x40cm) prior to and during the experiment. During the experiment, the front side of the cages was covered by carton to exclude external light. This was done to prevent a cage assigned to a control treatment being influenced by the light at night applied to an experimental cage in the same room. Birds were randomly assigned to the cages divided over two separate rooms and remained in the same cage for the entire experiment. Birds were kept under constant temperature (20 ºC) and had *ad libitum* access to water and food. Between October 2017 and January 2018 all the birds were exposed to artificial noise and nightlight during another experiment that addressed a different research question (*70*). However, all birds were exposed for the same number of days to light and noise also in the previous experiment, so we do not expect the results of this study to be biased. Daily health checks were performed to ensure the birds' welfare. After the end of the experiments the birds were released at their capture sites. The study was approved by an ethical committee (DEC-KNAW protocol NIOO 14.05, addendum 3 to MEV).

Experimental procedures

We tested for the impact of anthropogenic noise and light levels on the activity patterns of urban and forest birds by exposing each individual to four different treatments: control (C), artificial light at night (L), continuous acoustic noise (N) and both artificial light at night and continuous acoustic noise (L+N). All birds received the full-factorial treatment in a randomised but balanced order (ensuring that in each treatment week all four treatments were applied in a similar number). Each of the four treatment weeks consisted of five consecutive experimental treatment days followed by two days of recovery (no experimental light and/or noise).

Light treatment

Each cage was equipped with two types of lamps. For daylight, we used high frequency fluorescent lights emitting ± 1000 Lux at perch level (Activa 172, Philips, Eindhoven, The Netherlands (*57*)). These day lights went on at 08:00 and off at 17:30 (9.5:14.5 light/dark cycle). For the nightlight treatment we used a cool white LED light (Philips, Eindhoven, The Netherlands) switched on between 17:15 and 08:15, thus overlapping 15 minutes with the day lights, as in (*57*). The white LED light is broad-spectrum, but has a peak around 450 nm. For a full image of the spectrum please see (*59*). The night lights were set to 1.5 lux (measured at perch height before the start of the experiments for all lights), which is within the range of light levels songbirds are usually exposed to in light polluted areas (*22*).

Noise treatment

We developed a novel playback setup in order to avoid pseudo-replication and to keep the noise treatment similar to the light treatment. Instead of placing a speaker inside the cage (thereby creating an unwanted strong gradient), we connected our speaker setup to the outside of the cage, connected to the cart board and covered by sound absorbing foam to reduce

transfer to the neighboring cages. The speaker (an electro dynamical driver (EX 60 S, Visaton, Germany) was connected to one of five amplifiers (one Sony TA-F335R and four Renkforce SAP-702 amplifiers) and mp3 players (BaseTech BT-MP-100), placed in the center of the experimental room. We aimed to match noise conditions inside the cage to match the noise conditions observed in the field recorded inside a nest box situated ~100m away from a highway (*35*), very close to one of our study site. At these distances, noise is on average about 60 dB(A) SPL, more or less continuous and varies little in amplitude across the day (see (*35*) for spectrograms of these recordings). However, we also point out that this pattern might depend on the location, as previous studies also showed variation in noise amplitude and frequency during the 24 h (*71, 72*). We tried different noise types and settled for continuous pink noise (filtered white noise with a bias towards lower frequencies), as this best matched the nest box observations in terms of overall spectral shape (*35*). Using artificial, instead of real noise recording also made the noise treatment more similar to the light treatment (all birds receiving the same stimuli instead of using different sound replicates per bird). The noise was set to 60 dB SPL (as measured at the center of the cage with a Voltcraft SL-100 SPL meter, set to fast, max, and A-weighted). The baseline noise levels inside cages receiving no noise playback ranged from 38 – 45 dB SPL. Noise treatment started at 17:15 on the first day of a treatment week (the same time that night lights were turned on) and ended at 8:15 on the sixth day (thus at the same time that the night lights were turned off, see above).

Activity measures

Perch-hopping activity was recorded continuously throughout the experimental period (as described by (*58*)). Each cage contained a normal perch and a perch equipped with a micro switch. The switch was connected to a desktop PC with a custom-build program continuously

monitoring its position (developed by T&M Automation, Leidschendam, The Netherlands). The program scored whether the switch changed position in 30-second bins, logging a '1' (active) for each bin in which the switch moved position at least once, or a '0' (inactive) if nothing happened during those 30 seconds. The raw data on activity output (a 0 or 1 for every 30 seconds) was used to calculate the total activity, defined as the number of active minutes per 24 hours (from 17:00-17:00). Furthermore, the data was split in day and night activity (active minutes when the day lights were on and off, respectively). We also used a custom-build software program (ChronoShop 1.1, courtesy of Kamiel Spoelstra, see (*73*) and (*74*)) for previous applications of this software) to extract the activity onset and offset, calculated as the first and last moment (minutes relative to day lights on or off) of the day that the mean activity exceeded the average activity using a running mean of 20 minutes compared to the 24h average (following (*58*)). For activity offset we excluded the data for day 5, since on that day the experimental treatments ended in the afternoon. Finally, we excluded data for 24 (out of 660) treatment days, since on these days the perch connected to the micro switch was stuck or fell off.

Statistical analyses

We analysed the activity data using the statistical program R (version 3.4.4 (*75*)). We ran generalized linear mixed models (GLMMs) with a Poisson error structure using the *lme4* package (*76*). We ran five different models with total activity, daytime activity, nighttime activity, onset and offset of activity as response variables. We used random slopes model by including bird ID as random slope over treatment days). However, when we included offset of activity as response variable, the model presented singularity issues, so we decided to switch to a simpler random structure with only bird ID as random intercept. Moreover, the models

for total, daytime and nighttime activity were overdispersed, thus we included an observation-level random effect which efficiently corrected for this issue.

We used a backward selection process, starting off with initial models that contained a three-way interaction between light treatment, noise treatment and origin of the bird as fixed effect. In case of a significant three-way interaction we ran the analyses on the data for forest and urban birds separately. In a next step, we tested for significant main effects as well as all two-way interactions between origin, light, and noise treatment. We tested for significance of terms (main and interaction) by comparing models with and without the term of interest using likelihood ratio tests. Model assumptions were confirmed by visual inspection of the QQ plot of the residuals from the final model as well as by plotting residuals over fitted values to check for heteroscedasticity. We present statistics for the most important results in the text but include the complete outputs of the final models in the supplementary online materials. In the results section we also present back-transformed parameter estimates, usually as number of minutes, in order to facilitate the interpretation of the results. These estimates were obtained by computing predictions from the final model using the *predict.glmer* function in the package *lme4*. The predicted estimates were then back-transformed by exponentiating them to the scale of the response variable.

We followed up on any significant two-way interaction effect of noise and light exposure by comparing their estimated additive effect with the observed effect of combined exposure (interactive effect) following (*28*). To estimate the observed interactive effect we combined the full factorial parameter estimates of the model containing the significant interaction between light and noise. To estimate additive effects we summed parameter estimates from a reduced model containing only the two main effects of light and noise, but no interaction. We considered the combined impact of noise and light pollution on activity or timing measurements to be synergistic, or reinforcing, when the effect size was larger (either

positive or negative) than the effect size based on the estimated additive effects. We considered the impact antagonistic when the effect size was smaller (following (*28*)).

Results

Treatment effects on amount of activity

We found a significant three-way interaction effect of light, noise and origin on the total activity (χ^2 =17.9, p<0.001, suppl. Table 1). We then split the dataset between urban and forest birds and found for both populations a significant light*noise interaction (urban: χ^2 =32.5, p<0.001; forest: χ^2 =5.2, p=0.023; suppl. Table 1). Light alone had a significant effect on total activity of both urban and forest birds, increasing it by 24 min in urban birds and 12 min in forest birds compared to the control group. The combination of light and noise increases activity even more, by 39 mins in urban birds and by 29 in forest birds. Conversely, noise alone had an opposite effect, as it strongly reduced total activity in both urban birds (back-transformed estimate = - 50 min, χ^2 =9.4, p<0.001, suppl. table 1), and forest birds, although for the latter this effect was not significant (back-transformed estimate = - 23 min, χ^2 =1.01, p = 0.313). Thus, on one hand, noise enhanced the impact of light on total activity when we compared the observed effect with the estimated additive effect, showing synergy between the two stimuli (Fig. 3 and 5). On the other hand, light overruled the suppressing effect of noise on total activity (antagonistic effect).

To better understand the sources of variation in total activity, the next step we took was to split the activity data between daytime and nighttime, separated by the time at which day lights were turned on in the morning and off in the evening.

For *daytime activity* we found a significant 3-way interaction between light, noise and habitat of origin (χ^2 =17.7, p<0.001, Fig. 3B and Suppl. Table 3). In urban birds, the interaction of light and noise significantly affected daytime activity (χ^2 =19.1, p<0.001, Fig.

276 3B and Suppl. Table 3). Specifically, noise decreased daytime activity compared to control
277 birds by an average of 43 min per day (χ^2 =11.3, p<0.001, Fig. 3B and Suppl. Table 3), while
278 light had no significant impact on its own (χ^2 =0.5, p=0.489, Fig. 3B and Suppl. Table 3). The
279 effect of noise on daytime activity was reduced when combined with light exposure (only 20
280 min reduction compared to control) and when compared to the estimated additive effect (23
281 min, Fig. 3B and Fig. 5), showing antagonism between the two stimuli. Conversely, in forest
282 birds, noise and light both significantly decreased daytime activity (light: back-transformed
283 estimate = - 24 min, χ^2 = 19.3, p=<0.001; noise: back-transformed estimate = -11 min, χ^2 =
284 4.1, p=0.043; Fig. 3B and Suppl. Table 3), independently of whether they were presented
285 alone or in combination (interaction light*noise: χ^2 = 3.3, p=0.070, Fig. 2B and Suppl. Table
286 3). Therefore, for forest birds the interactive effect did not differ from the additive effects of
287 noise and light at night (Fig. 5).

288 For *nighttime activity* we also detected a significant three-way interaction between
289 light, noise and origin (χ^2 = 14.8, p=0.002, Fig. 3C and Suppl. Table 4). The interaction
290 between light and noise was retained when focusing on the urban birds (χ^2 = 9.4, p=0.002,
291 Fig. 3C and Suppl. Table 44), as the combination of light and noise increased nighttime
292 activity by 30 minutes. This effect was very similar to the effect of light alone, which caused
293 urban birds to be on average over 26 min more active at night (χ^2 = 243.7, p<0.001, Fig. 3C
294 and Suppl. Table 4). Conversely, when exposed to noise urban birds had a lower amount of
295 nocturnal activity compared to control treatment, although the effect size was small (back-
296 transformed estimate = - 3 min, χ^2 = 4.9, p=0.027, Fig. 3C and Suppl. Table 4). Thus, when
297 comparing estimated additive effects of light and noise with the observed interactive effect of
298 the two stimuli, we find that light and noise had a synergistic effect (Fig. 5). For forest birds,
299 we only found a significant positive effect of light on nighttime activity (back-transformed

estimate = 35 min, χ^2 = 294.6, p<0.001, Fig. 3C and Suppl. Table 4) and neither an effect of noise nor a significant interaction (Fig. 3C and Suppl. Table 4).

Treatment effects on timing of activity

The onset of activity was mainly influenced by light (χ^2 = 104.6, p<0.001, Fig. 4A and Suppl. Table 5), while noise and habitat of origin had no effect on this trait (Fig. 4A and Suppl. Table 7). The effect size was large: in both the light and the light + noise groups birds started to be active on average 55 mins before lights on compared to both the control and the noise groups (Fig. 4A).

The offset of activity was affected by light, noise and origin in a three-way interaction (χ^2 = 11.3, p=0.010, Fig. 4B and Suppl. Table 6). In forest birds light delayed offset of activity by 19 mins (χ^2 = 29.7, p<0.001, Fig. 4B and Suppl. Table 6), independently of whether it was presented alone or in combination with noise (light*noise interaction: χ^2 = 0.4, p=0.509, Fig. 4B and Suppl. Table 6). Conversely, offset of activity was not affected by any of the explanatory variables in urban birds (Suppl. Table 6).

Synergistic, antagonistic or overruling effects of noise and light

We found that noise and light exposure had a synergistic effect on total activity for both the urban and forest birds (Fig. 5). Furthermore, we found for urban birds that noise and light had a synergistic effect on nighttime activity and an antagonistic effect on daytime activity (Fig. 5). For the timing of activity, we found that light at night largely overruled noise in driving changes in the time of onset and offset of activity (Fig. 4).

Discussion

Understanding how human activity shapes activity of wild animals is a global research challenge (*77*). In recent years, the impact of artificial light at night and anthropogenic noise have received much attention. However, there is considerable debate about the relative importance of each of these stimuli in affecting activity of wildlife, mostly due to a lack of experimental work. Here we show, via an experimental manipulation, that both light and noise can affect activity patterns when presented alone, but in opposite ways: light increased activity, particularly at night, while noise reduced it, particularly at daytime. When the two stressors were combined, we found a complex pattern of interactive effects, which differed depending on the origin of birds. In general, birds caught in forested areas showed no synergistic or antagonistic response to light and noise, except on their total activity (though the effect tended to be smaller compared to the urban birds). Conversely, when urban birds were exposed to a combination of light and noise, they showed a synergistic response of increased nighttime activity and an antagonistic response of reduced daytime activity. Activity onset and offset were generally affected only by light at night in all birds, although the effects were much weaker for the offset of activity. Below we break down these results and offer an interpretation of the mechanisms that might underlie such effects.

The effect of light on activity patterns

The positive effect of light on total daily activity was mainly explained by increased nighttime activity. Indeed, light advanced activity onset of up to two hours in some individuals, and consequently increased nighttime activity by an average of one hour per night. This result is in line with previous studies that have shown that artificial light at night can increase nocturnal restlessness as well as foraging behaviour in great tits and other diurnal bird species (*78–80*). These effects are likely to come through the impact of light on physiological processes such as clock gene expression and melatonin production (*56*, *58*). Vice versa,

exposure to light at night, both separately and in combination with noise, reduced activity levels at daytime. Such result might be a consequence of birds resting during the day to compensate for the increased amount of activity during the night. However, an alternative hypothesis is that birds exposed to light at night have phase-advanced their circadian rhythm of activity, resulting in a higher proportion of the daily activity overlapping with the nighttime. Our data seems to suggest the first hypothesis is more likely. Indeed, the time of daily peak of activity does not differ between treatment groups, as shown in Figure 2, discarding the hypothesis that the daily rhythm of activity was simply phase-shifted by light at night. In a previous experiment that used a comparable set-up, we showed that birds exposed to light at night spent more energy over a 24-h period compared to control birds (*70*). Thus, increased nocturnal activity due to sensory pollution might drive up energy costs, and resting during the daytime hours could be a way to minimize such costs, if birds would be able to habituate to sensory stress exposure. However, these experiments were conducted in highly controlled captive environments. While on one hand this allows to precisely quantify the single and interactive effects of light and noise, on the other hand we do not know whether these effects would persist in the wild. Future studies should directly test this hypothesis in more realistic field settings where such decisions might be modulated by other environmental factors such as the risk of starvation and predation.

The effect of noise on activity patterns

Anthropogenic noise on its own reduced activity, in particular during the day. Furthermore, urban birds reduced their night, dusk and dawn activity when exposed to noise only. These results can be explained in different ways. Birds exposed to noise may be distracted or confused, which could in turn lead to increased levels of circulating stress hormones and associated changes in activity. Chronic and acute exposure to noise has been shown to induce

a stress response and to lead to increased levels of corticosterone in various bird species (*48, 81, 82*). Stressed birds may generally show less activity or take less risk and be therefore less active.

Alternatively, birds exposed to noise may suffer from masking of conspecific acoustic cues. Despite the isolation foam placed outside of the cages, control birds in our experiment may still have been able to hear conspecifics in neighboring cages and may have responded to these acoustic cues by increasing their activity. Conversely, the activity of birds in the noise treatment group may have been less affected by the activity of their peers in neighboring cages. However, birds might have also heard noise from nearby experimental cages, which could have further affected their activity. Our data does not allow to discriminate between these alternative explanations. However, we stress that we used a within-individual repeated measure design, so all birds received the same treatments albeit at different treatment weeks. Moreover, the fact that we see differences between groups suggest that any potential confound is smaller than the treatment effects.

Explanations for synergistic or antagonistic effects of light and noise

Light at night had a strong positive effect on daily activity levels, particularly during the night. Conversely, noise had an opposite, negative effect on activity levels, particularly during daytime and in urban birds. However, when light and noise were presented together, most birds responded as if they were exposed to higher light levels, showing increased total and night activity compared to birds exposed to light alone. This highlights on one hand the overriding effect of anthropogenic light over noise, and on the other hand the synergistic, reinforcing effect of noise on light. While the overriding effect of light on noise is not really surprising given that photoperiod is presumably a far stronger driver of diurnal activity

patterns than noise, the synergistic effect is harder to explain and thus requires further investigation.

Population differences in activity traits and their sensitivity to light and noise

We found strong differences between urban and forest birds in their response to the different treatments. For instance, urban birds seemed to be more sensitive to noise: both daily and nocturnal activity were reduced in this group of birds when they were exposed to noise alone, compared to the control treatment. This result is in line with two recent studies that also showed higher short-term sensitivity to noise in urban compared to rural songbirds (*69, 83*). Moreover, our previous experiment that used the same birds tested in this study has shown that forest birds increased their night activity in response to white LED light exposure more strongly than urban birds did. Overall, these results suggest that urban and forest birds may differ in their sensitivity to light and noise. Future studies should investigate whether this pattern might depend on the species or on the history of colonization of urban areas.

On top of these differences in sensitivity to light and noise, we also found that control urban and forest birds differed in their activity traits. For instance, urban birds in the control group started their activity on average 15 minutes earlier than control forest birds. Moreover, the amount of activity around dusk and the time of activity offset also differed between control forest and urban birds. Several field studies have shown that in different songbird species urban individuals start their activity earlier than forest conspecifics (*63, 66, 84*). In particular, previous work on European blackbirds (*Turdus merula*) has shown that urban blackbirds have a faster endogenous circadian clock compared to forest ones, and suggested that this could be a mechanistic basis for earlier awakening time in urban areas (*85*). We have recorded circadian period length in constant conditions in a limited subsample of our birds and found that urban individuals showed a strong tendency to have a shorter period length

than forest ones, although this was not significant (Dominoni et al *unpublished data*). We speculate that the slightly faster clock of urban great tits might be responsible for the differences in onset time between the control birds of our experiments, but more evidence is needed to prove this hypothesis.

At this stage, it is unclear whether any differences in activity traits or sensitivity to light and noise in urban and forest birds have a genetic basis or represent plastic phenotypic responses. Our birds were wild caught adult individuals, thus it is not possible to disentangle genetic vs non-genetic effects (including developmental ones) on circadian timing and sensitivity to anthropogenic stressors between urban and forest individuals. A previous study on European blackbirds has revealed no genetic differences in a circadian clock gene (*CLOCK*) between urban and forest birds (*86*). However, activity patterns are likely to be polygenic traits. Future studies could use whole-genome sequencing and/or common-garden experiments to fill this gap.

Conclusions

We have shown that artificial light at night and noise interact and produce complex effects on activity patterns of a model songbird species. On the one hand, light at night may override a daytime effect of noise, whereas on the other hand, continuous noise exposure may enhance the effect of light during the night as well as around dusk and dawn, more than the simple addition of the single effects of these stimuli. Thus, our results point to multisensory pollution being a considerable threat to wildlife and stress the importance of including both these anthropogenic stressors in future assessments of the ecological effects of urbanisation and human activity.

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Author contributions

DMD, JAHS, MEV, WH conceived the study and designed the experiment. DMD caught the birds in the wild. JAHS with the help of DMD and WH performed the experiment. JAHS analysed the data with input from DMD and WH. DMD wrote the paper with help from all co-authors. All authors agreed on the final version of the manuscript.

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Figure and legends

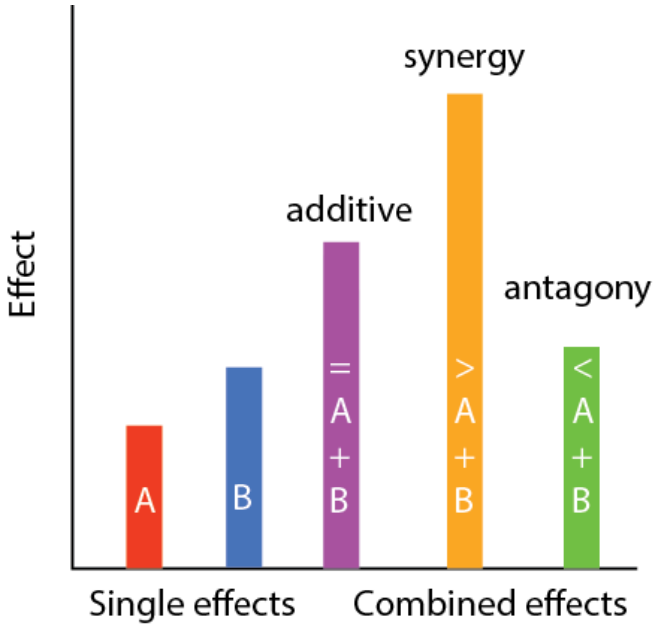

Figure 1. Predicted interactive effects of multiple sensory pollutants. Different linear and non-linear predictions are shown to demonstrate the potentially complex outcome of combining multiple sensory pollutants. The combined effect (AB) of two sensory pollutants (e.g. noise and light) can be additive (AB=A+B) or show a significant interaction. In the latter case, the observed pattern can be antagonistic (AB< A+B) or synergistic (AB>A+B).

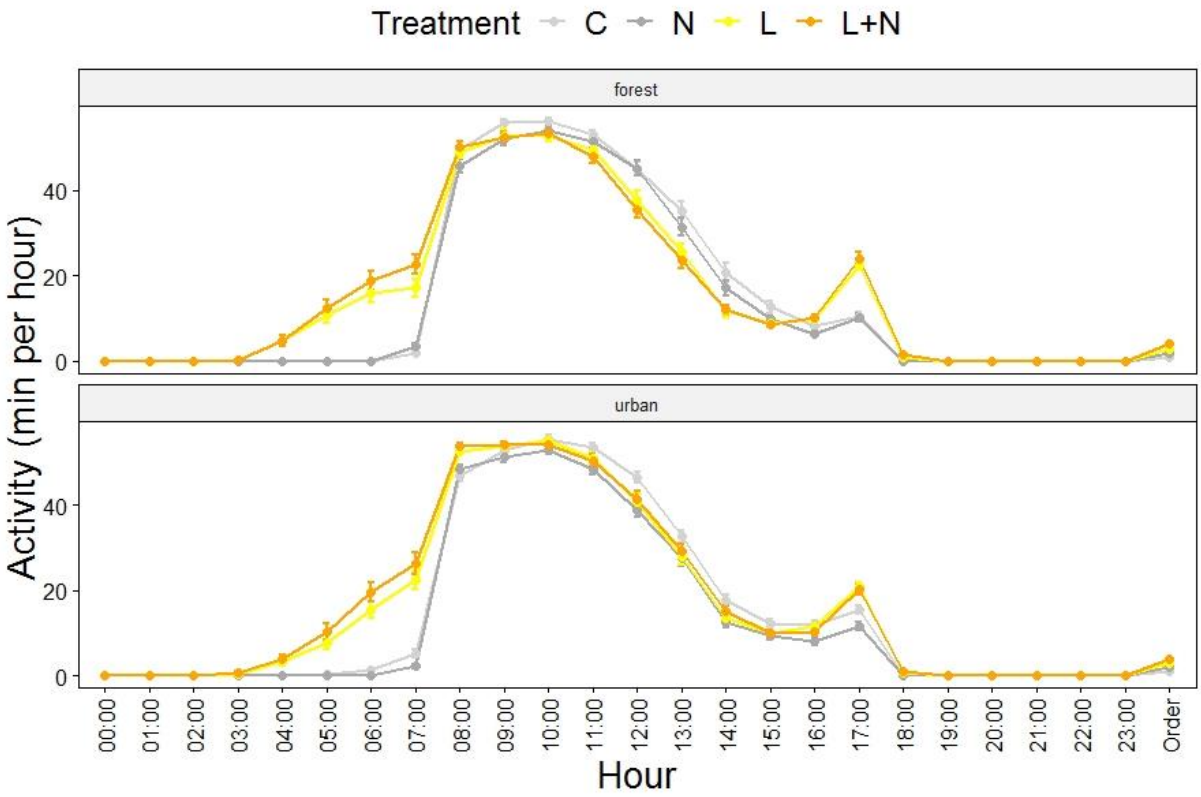

Figure 2. Hourly activity of male great tits exposed to control (C), noise (N), light (L) light plus noise (L+N) conditions. The raw activity data (mean ± S.E) is plotted for forest (upper) and urban (lower) birds separately.

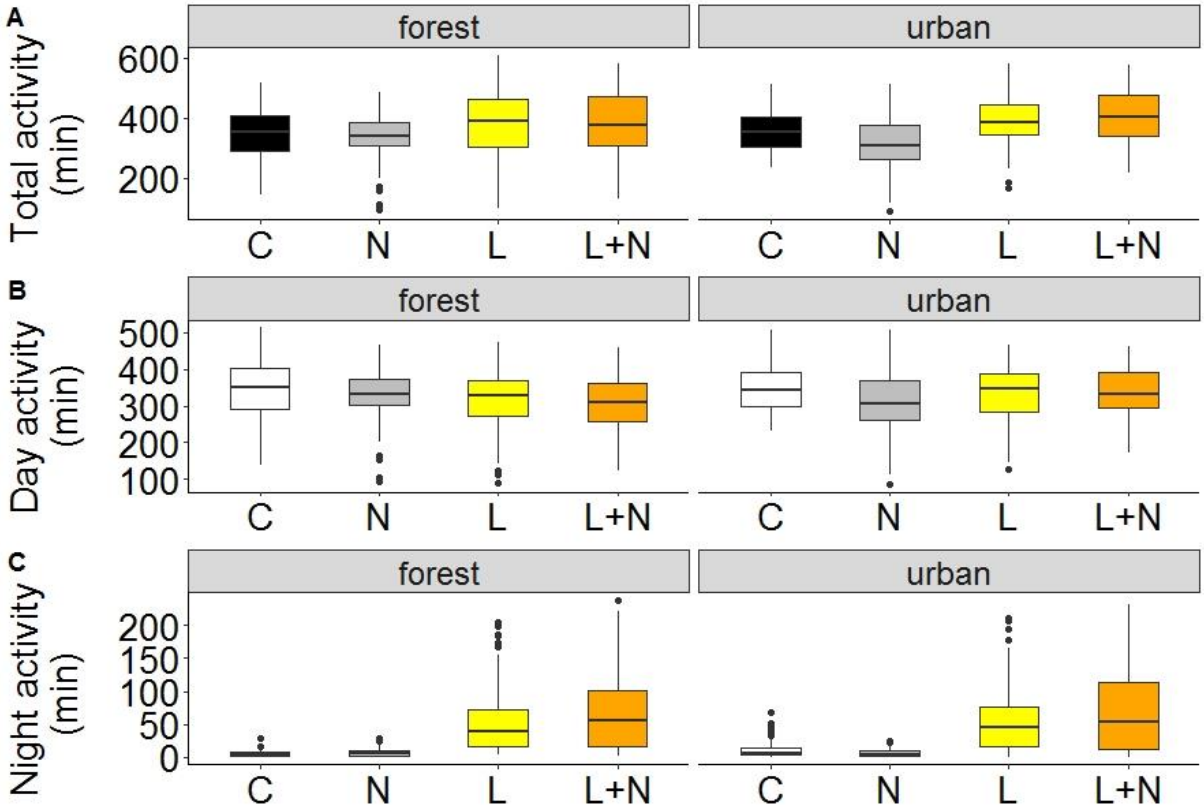

Figure 3. Effects of light, noise and their interaction on the amount of activity during the whole 24h (A), daytime (B) or nighttime (C). The control (C), noise (N), light (L) and light plus noise (L+N) treatment groups are displayed for forest (left) and urban (right) birds separately. For statistics see supplementary tables 1-3.

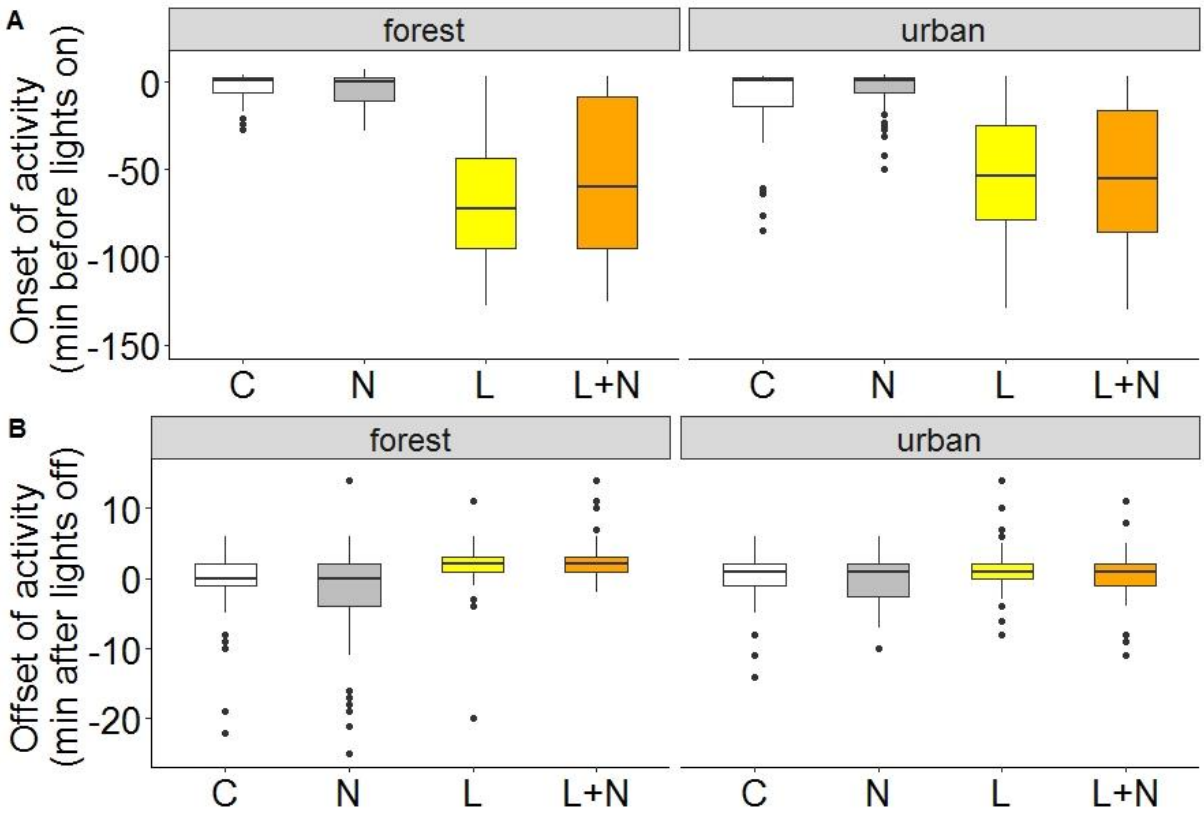

Figure 4. Effects of light, noise and their interaction on timing of dawn (A) and dusk (B) activity. The control (C), noise (N), light (L) and light plus noise (L+N) treatment groups are displayed for forest (left) and urban (right) birds separately. For statistics see supplementary tables 5-6. Activity onset and offset were calculated for each bird for each day with the software Chronoshop (Kamiel Spoelstra), then averaged over treatment days for each treatment.

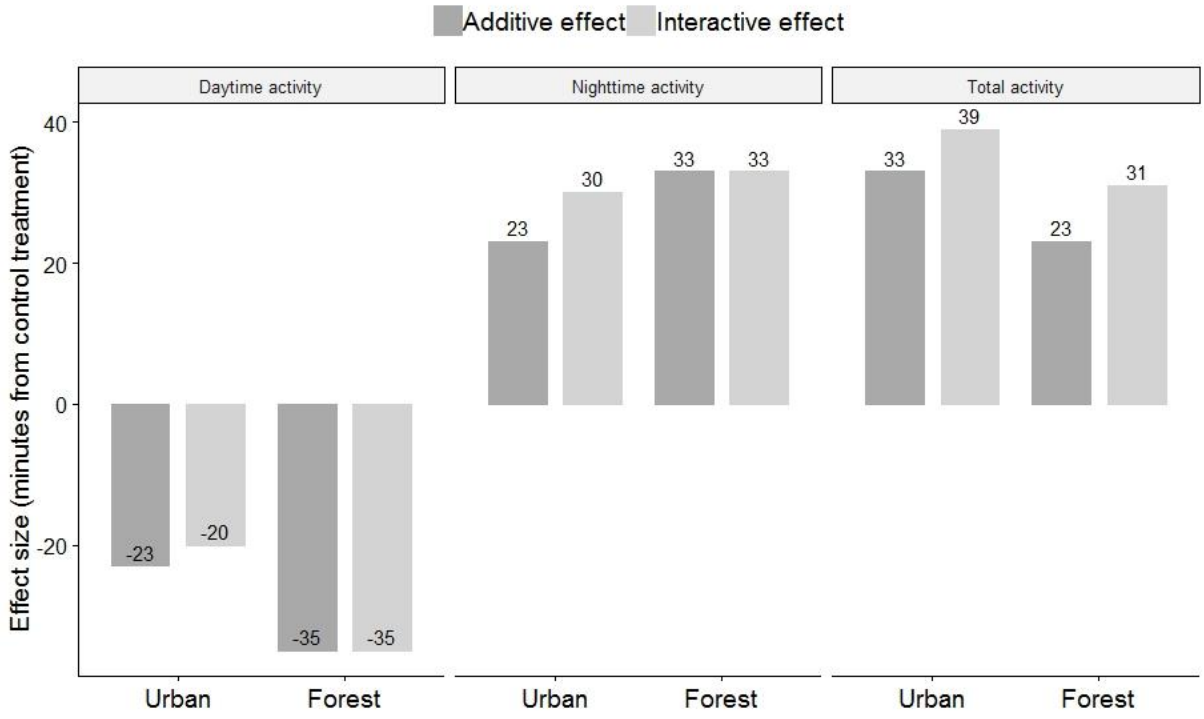

Figure 5. Effect sizes based on predicted additive effects of light and noise versus their observed interactive effect on great tit activity traits. Effect sizes were calculated from back-transformed parameter estimates of Poisson GLMMs run without (additive effects) or with (interactive effects) the light*noise interaction. Labels on top of each bar depicts effect size (difference from control treatment) in minutes.